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<p><b>(21) International Application Number:</b> <b>T/CA98/01065</b></p> <p><b>(22) International Filing Date:</b> <b>13 November 1998 (13.11.98)</b></p> <p><b>(30) Priority Data:</b>  <b>60/065,793</b>                    <b>14 November 1997 (14.11.97)</b>    <b>US</b> </p> <p><b>(71) Applicant (for all designated States except US):</b> CONNAUGHT LABORATORIES LIMITED [CA/CA]; 1755 Steeles Avenue West, North York, Ontario M2R 3T4 (CA).</p> <p><b>(72) Inventor; and</b>  <b>(75) Inventor/Applicant (for US only):</b> PARRINGTON, Mark [CA/CA]; 45 Main Street, Bradford, Ontario L3Z 1Z4 (CA).</p> <p><b>(74) Agent:</b> STEWART, Michael; 6th floor, 330 University Avenue, Toronto, Ontario MSG 1R7 (CA).</p>		<p><b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b>  <i>With international search report.  Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p><b>(54) Title:</b> ALPHA VIRUS VECTORS</p> <p><b>(57) Abstract</b></p> <p>A modified alphavirus expression vector is provided wherein at least one optimal heterologous splice site is introduced to the alphavirus replicon to prevent aberrant splicing of the alphavirus, which may be Semliki Forest virus following administration of the vector to a host.</p>		

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TITLE OF INVENTIONALPHAVIRUS VECTORS

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FIELD OF INVENTION

The present invention relates to the field of DNA vaccines and is particularly concerned with modified alpha virus vectors for use in such vaccines.

BACKGROUND OF THE INVENTION

10 Semliki Forest virus (SFV) is a member of the Alphavirus genus in the Togaviridae family. The mature virus particle contains a single copy of a ssRNA genome with a positive polarity that is 5'-capped and 3'-polyadenylated. It functions as an mRNA and naked RNA  
15 can start an infection when introduced into cells. Upon infection/transfection, the 5' two-thirds of the genome is translated into a polyprotein that is processed into the four nonstructural proteins (nsP1 to 4) by self cleavage. Once the ns proteins have been synthesized  
20 they are responsible for replicating the plus-strand (42S) genome into full-length minus strands (ref. 14). These minus-strands then serve as templates for the synthesis of new plus-strand (42S) genomes and the 26S subgenomic mRNA (ref. 1 - Throughout this application,  
25 various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification. The disclosures of these references are hereby incorporated  
30 by reference into the present disclosure). This subgenomic mRNA, which is colinear with the last one-third of the genome, encodes the SFV structural

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proteins. In 1991 Liljestrom and Garoff (ref. 2) designed a series of expression vectors based on the SFV CDNA replicon. These vectors had the virus structural protein genes deleted to make the way for heterologous inserts, but preserved the nonstructural coding region for production of the nsP1 to 4 replicase complex. Short 5' and 3' sequence elements required for RNA replication were also preserved. A polylinker site was inserted downstream from the 26S promoter followed by translation stop sites in all three frames. An SpeI site was inserted just after the 3' end of the SFV CDNA for linearization of the plasmid for use in vitro transcription reactions.

Injection of SFV RNA encoding a heterologous protein have been shown to result in the expression of the foreign protein and the induction of antibody in a number of studies (refs. 3,4). The use of SFV RNA inoculation to express foreign proteins for the purpose of immunization would have several of the advantages associated with plasmid DNA immunization. For example, SFV RNA encoding a viral antigen may be introduced in the presence of antibody to that virus without a loss in potency due to neutralization by antibodies to the virus. Also, because the protein is expressed *in vivo* the protein should have the same conformation as the protein expressed by the virus itself. Therefore, concerns about conformational changes which could occur during protein purification leading to a loss in immunogenicity, protective epitopes and possibly immunopotentiation, could be avoided by plasmid DNA immunization.

In WO95/27044, the disclosure of which is incorporated herein by reference, there is described the use of alphavirus cDNA vectors based on cDNA complementary to the alphavirus RNA sequence. Once 5 transcribed from the cDNA under transcriptional control of a heterologous promoter, the alphavirus RNA is able to self-replicate by means of its own replicase and thereby amplify the copy number of the transcribed recombinant RNA molecules.

10

SUMMARY OF THE INVENTION

The present invention is concerned with modifications to the alphavirus cDNA vectors described in the aforementioned WO 95/27044 to permit enhanced replication of the alphavirus. In the present 15 invention, a heterologous splice site is introduced into the alphavirus replicon sequence, particularly that of Semliki Forest virus (SFV).

Accordingly, in one aspect, the present invention provides an expression vector comprising a DNA molecule 20 complementary to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions which are essential for replication of the said alphavirus RNA, and further comprises a heterologous DNA sequence 25 capable of expression in a suitable host, such as a human or animal host, said heterologous DNA sequence being inserted into a region of the DNA molecule which is non-essential to replication thereof, and the DNA molecule being placed under transcriptional control of 30 a promoter sequence functional in said animal or human host, wherein at least one heterologous splice site is

provided in the DNA molecule to prevent aberrant RNA splicing of the alphavirus.

The alphavirus molecule is a large molecule and, accordingly, there is a high probability of cryptic splice sites, thereby impairing the replication of the alphavirus and hence its ability to express the heterologous DNA is impaired. By introducing the at least one optimal heterologous splice site in accordance with the present invention into the alphavirus replicon sequence, any splicing is likely to be directed at the heterologous splice site rather than any cryptic splice sites, restores the function of the SFV replicon when removed, and may improve transport of RNA from the nucleus (ref. 6).

In the constructs provided herein, the promoter is placed upstream of the 5'-end of the alphavirus sequence, such that the resultant transcript has an authentic 5'-end, which is required for the efficient replication of the alphavirus RNA replicon.

In addition, there may be provided at the 3'end of the Semliki Forest virus segment, a hepatitis delta virus ribozyme sequence to ensure proper *in vivo* cleavage at the 3'-end of the sequence. Any other convenient sequence may be employed to achieve this effect.

The heterologous splice site sequence may be provided by the nucleotide sequence of the rabbit  $\beta$ -globin intron II, as described in reference 5. Such heterologous splice site sequence may be inserted into the complement sequence at any convenient location which generates perfect splice junctions. This

precludes replication of the alphavirus, unless it is authentically removed by splicing..

I have identified five suitable sites in the SFV replicon, which are contained within an EcoRV-SpeI fragment of the replicon which is 8010 bp in length (Fig. 3). The first such site is a Ppu-MI site, at position 2719 within the EcoRV-SpeI fragment.

In constructing the modified vectors provided herein, the EcoRV-SpeI fragment is cut with Ppu-MI at position 2719 and made blunt-ended with Mung Bean nuclease, which removes three bases from the SFV sequence. A blunt-ended β-globin II intron, which is 536 bp long, is ligated into the site and replaces the missing three bases with sequence added to the 3'-end of the β-globin intron sequence (Fig. 1).

The other four suitable sites for insertion of the Intron are the PvuII sites at bp 2518, 3113, 6498 and 6872 of the EcoRV-SpeI fragment. Insertion of the Intron is achieved by cutting with PvuII (a blunt end cutter) and the blunt-ended β-globin II intron sequence (Fig. 2) is ligated into one or more of these sites.

In a further aspect of the present invention, there is provided a cloning vector suitable for expression in a host cell of an heterologous DNA sequence, which comprises a DNA molecule complementing to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions and has a cloning site for insertion therein of a heterologous DNA sequence capable of expression in a host cell, said cloning site being located in a region of the DNA molecule which is

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non-essential to replication thereof; a promoter sequence functional in said host cell and transcriptionally controlling said DNA molecule, said promoter sequence being placed upstream of the 5'-end  
5 of the DNA molecule such that the resultant transcript had an authentic 5' end; at least one heterologous splice site provided in the complement of the DNA molecule to generate perfect splice junctions in the alphavirus in order to prevent aberrant splicing and an  
10 additional DNA sequence at the 3'-end of the DNA molecule to direct proper *in vivo* cleavage at the 3'-end of the reactant mRNA transcript.

**BRIEF DESCRIPTION OF DRAWINGS**

Figure 1 shows the DNA sequence of the β-globin  
15 intron II including three additional nucleotides at the 3'-end thereof (SEQ ID No:1);

Figure 2 shows the DNA sequence of the β-globin  
intron II (SEQ ID No:2);

Figures 3A to 3C show the DNA sequence of the  
20 EcoRV-SpeI fragment of Semliki Forest virus replicon  
(SEQ ID No:3);

Figures 4A to 4D show the DNA sequence of the pSFV  
link (SEQ ID no: 4) prepared as illustrated in Figure  
5;

25 Figure 5 shows construction of pSFVlink (11060 bp)  
from pSFV1 using a linker sequence (SEQ ID nos: 5,6);

Figures 6A to 6D show the nucleotide sequence of  
plasmid pMP76 (SEQ ID no: 11, prepared as illustrated  
in Figures 8A to 8D;

30 Figure 7 illustrates subsections of plasmid pSFV  
link (see Figure 5);

Figure 8A to 8D show the construction of plasmid pMP76 from plasmids pMP53, pMP70, pMP47, pMP55 and pMP71;

Figures 9A to 9B show the construction of plasmids 5 pMP53, pMP54 and pMP55 from plasmid pMP52;

Figure 10 shows the construction of plasmid MP52 from pUC19 using a linker sequence (SEQ ID no: 7,8);

Figures 11A to 11B show the construction of 10 plasmids pMP46, pMP47 and pMP70 from pUC19 and fragment from pSFV link, prepared as seen in Figure 7; and

Figures 12A to 12B show the construction of plasmid pMP71 from plasmid pCMV3.

#### GENERAL DESCRIPTION OF INVENTION

15 As discussed above, the present invention provides a modified alphavirus DNA. The alphavirus preferably is Semliki Forest virus. In particular, the present invention provides a cloning vector for heterologous gene expression in a host, such as an animal or human.

20 The promoter sequence may comprise a promoter of eukaryotic or prokaryotic origin. Suitable promoters are the cytomegalovirus immediate early promoter (pCMV), although other promoters, such as the Rous sarcoma virus long-terminal repeat promoter (pRSV), 25 since, in the case of these and similar promoters, transcription is performed by the DNA-dependent RNA polymerase of the host cell. Additionally, the SP6, T3 or T7 promoters can be used, provided that the cell has first been transformed with genes encoding SP6, T3 or 30 T7 RNA polymerase molecules which are either inserted into the chromosome or remain episomal. Expression of

these (SP6, T3, T7) RNA polymerase-encoding genes is dependent on the host cell DNA-dependent RNA polymerase.

The heterologous DNA insert may comprise the 5 coding sequence for a desired product, which may be a biologically active protein or polypeptide, for example, the heterologous DNA insert may code for HIV sequences, e.g., an immunogenic or antigenic protein or polypeptide, or a therapeutically active protein or 10 polypeptide. The heterologous DNA may also comprise additional sequences, such as a sequence complementary to an RNA sequence which is a self-cleaving ribozyme sequence.

The DNA vectors provided herein may be 15 administered to a host, including a human host, for *in vivo* expression of the heterologous DNA sequence, in accordance with a further aspect of the invention, in order to generate an immune response in the host, which may be a protective immune response. The DNA vectors 20 may be further formulated into immunogenic compositions for such administration.

#### BIOLOGICAL DEPOSITS

Certain vectors that contain the Semliki Forest 25 virus replicon and referred to herein have been deposited with the American Type Culture Collection (ATCC) located at 10801 University Boulevard, Manassas, VA 20110-2209, U.S.A., pursuant to the Budapest Treaty and prior to the filing of this application.

30 Samples of the deposited plasmids will become available to the public upon grant of a patent based

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upon this United States patent application and all restrictions on access to the deposits will be removed at that time. Non-viable deposits will be replaced.

The invention described and claimed herein is not to be  
5 limited in scope by plasmids deposited, since the deposited embodiment is intended only as an illustration of the invention.

Deposit Summary

	<u>Plasmid</u>	<u>ATCC Designation</u>	<u>Date Deposited</u>
10	pMP76		

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can  
15 be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as  
20 circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein  
25 biochemistry and immunology used but not explicitly described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

EXAMPLE 1

This Example describes the construction of plasmid pMP76 as outlined in Figures 5, 7, 8A, 8B, 8C, 8D, 9A, 9B, 10, 11A, 11B, 12A and 12B.

5 Plasmid pSFV link was created by restricting plasmid pSFV1 (Gibco) with BamHI. This plasmid was then ligated with a linker (SEQ ID no: 5 and 6) to produce plasmid pSFV link (Figures 4A to 4D, Figure 5).

Some of the SFV replicon fragments were subcloned  
10 by restricting pSFVlink with EcoRV and SpeI and isolating the 890bp EcoRV-SpeI fragment. This fragment was then restricted with EcoRI and the 1906bp EcoRV-EcoRI, the 1578bp and 3627bp EcoRI-EcoRI and the 899bp EcoRI-SpeI fragments isolated (Fig.7).

15 The 1909bp EcoRV-EcoRI SFV fragment was cloned into EcoRV-EcoRI restricted plasmid pMP52 to produce plasmid pMP53 (Fig.9A). The 899bp EcoRI-SpeI SFV fragment was cloned into EcoRI-SpeI restricted pMP52 to produce pMP54 (Fig.9A). Plasmid pMP54 was then  
20 restricted with SpeI and made blunt-ended with Mung Bean nuclease. The plasmid was then restricted with BglIII, dephosphorylated and ligated to the hepatitis delta virus ribozyme linker (SEQ ID nos. 9 and 10), that had been phosphorylated, to produce pMP55 (Fig.  
25 9B).

Plasmid pMP52 was created by ligating a linker (SEQ ID nos:7,8), into the EcoRI site of pUC19 (Fig.10).

The 1578bp EcoRI-SFV fragment was cloned into  
30 the EcoRI site of pUC19, to produce pMP46 (Fig.11A). This plasmid was then restricted with PpuM1 and made

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blunt-ended with Mung Bean nuclease. The rabbit  $\beta$ -globin intron II PCR fragment (Fig.1) was made blunt-ended with Mung Bean nuclease, phosphorylated and ligated to the PpuMI restricted pMP46 to produce 5 plasmid pMP70 (Fig.11B).

The 3627bp EcoRI SFV fragment was cloned into the EcoRI site of pUC19 to produce pMP47 (Fig.11A).

Plasmid pCMV3, which contains the CMV promoter, Intron A sequence, BGH poly A sequence and 10 SU40 poly A sequence, was restricted with NdeI and EcoRV. The 3191bp NdeI-EcoRV fragment was isolated and dephosphorylated. The 1321bp NdeI-EcoRV fragment was isolated and restricted with SacI. The NdeI-SacI fragment of 334bp was isolated (Fig.12A). The isolated 15 SacI-EcoRV PCR fragment containing the 5'-end of SFV was ligated to the previously isolated 334bp NdeI-SacI fragment and the 3191bp NdeI-EcoRV fragment to produce pMP71 (Fig.12A and 12B).

Plasmid pMP53 was then restricted with EcoRI 20 and BamHI and ligated to the isolated and dephosphorylated 2151bp EcoRI fragment from pMP70 (Fig.8A). This ligation was then restricted with EcoRV and the 4057bp EcoRV-EcoRI fragment purified(Fig.8A).

Plasmid pMP47 was restricted with EcoRI and 25 the 3627bp EcoRI fragment isolated and dephosphorylated (Fig.8B). Plasmid pMP55 was then restricted with BglII, dephosphorylated and restricted with EcoRI. The 985bp EcoRI-BglII fragment was isolated and ligated to the previously isolated EcoRI fragment from pMP47 30 (Fig.8B). The ligation reaction was then

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phosphorylated and the 4612bp EcoRI-BglII fragment isolated.

Plasmid pMP71 was restricted with EcoRV and BamHI then dephosphorylated. This fragment was used in a 3-way ligation with the previously isolated 4612bp EcoRI-BglII fragment from pMP47 and pMP55, and the 4057bp EcoRV-EcoRI fragment from pMP53 and pMP70, to produce pMP76 (Figs. 8B and 8C).

The 5' end of the SFV replicon was produced by PCR amplification of pSFV1 using primers SFV-5'-3 having the sequence

5'-ATCTATGAGCTCGTTAGTGAACCGTATGGCGGATGTGTGACATACA-3'

and EcoR-SPE having the sequence

5'-TCCACCTCCAAGGATATCCAAGAGATGAGTGTG-3' (SEQ ID no: 9 and SEQ ID no: 10 respectively) between the CMV promoter and the 5' end of the SFV replicon. The resulting PCR fragment was restricted with SacI and EcoRV (Fig. 13; SEQ ID no: 11) and the fragment isolated.

#### SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides a modified alphavirus-based expression vector wherein at least one optimal splice site is introduced to the alphavirus replicon to prevent aberrant splicing of the alphavirus genome; and improve transport of RNA out of the nucleus. Modifications are possible within the scope of the invention.

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CLAIMS

1. An expression vector, comprising a DNA molecule complementary to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions which are essential for replication of the said alphavirus RNA and further comprises a heterologous DNA sequence capable of expression in a host, said heterologous DNA sequence being inserted into a region of the DNA molecule which is non-essential to replication thereof, and the DNA molecule being placed under transcriptional control of a promoter sequence functional in said host, wherein at least one heterologous splice site is provided in the DNA molecule to prevent aberrant RNA splicing of the alphavirus.
2. The vector of claim 1 wherein said promoter is placed upstream of the 5'-end of the DNA molecule such that the resultant transcript has an authentic 5'-end.
3. The vector of claim 2 wherein said promoter is the cytomegalovirus immediate early promoter.
4. The vector of claim 1 which further comprises an additional DNA sequence at the 3'-end of the DNA molecule to direct proper *in vivo* cleavage at the 3'-end of the DNA molecule.
5. The vector of claim 4 wherein said additional DNA sequence comprises a hepatitis delta ribozyme sequence.
6. The vector of claim 1 wherein the heterologous splice site sequence is provided by the DNA sequence of the rabbit  $\beta$ -globin intron II.
7. The vector of claim 6 wherein the heterologous splice site sequence is inserted into the DNA molecule

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at a location which generates perfect splice junctions and restores the function of the SFV replicon when removed.

8. The vector of claim 1 wherein the alphavirus is a  
5 Simliki Forest virus.

9. A cloning vector suitable for expression in a host cell of an heterologous DNA sequence, which comprises:  
a DNA molecule complementing to at least part of an alphavirus RNA genome, which DNA molecule comprises  
10 the complement of the complete alphavirus RNA genome regions and has a cloning site for insertion therein of a heterologous DNA sequence capable of expression in a host cell, said cloning site being located in a region of the DNA molecule which is non-essential to  
15 replication thereof;

a promoter sequence functional in said host cell and transcriptionally controlling said DNA molecule, said promoter sequence being placed upstream of the 5'-end of the DNA molecule such that the resultant  
20 transcript had an authentic 5' end;

at least one heterologous splice set provided in the complement of the DNA molecule to permit aberrant RNA splicing of one to generate perfect splice junctions in the alphavirus; and

25 an additional DNA sequence at the 3'-end of the DNA molecule to direct proper *in vivo* cleavage at the 3'-end of the reactant RNA molecule.

10. The cloning vector of claim 9 wherein said heterologous splice set is provided by the DNA sequence  
30 of the rabbit  $\beta$ -globin intron II.

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11. The cloning vector of claim 9 wherein said additional sequence comprises a hepatitis delta ribozyme sequence.
12. The cloning vector of claim 8 wherein the alphavirus is a Semliki Forest virus.
13. The cloning vector of claim 8 which has the identifying characteristics of plasmid pMP76 shown in Figure 8D.
14. The cloning vector of claim 8 having SEQ ID no: 10 11.

**FIG.1**

Nucleotide Sequence of the  $\beta$ -globin intron II with the 3' SFV bases

gtgaggttgg ggacccttga ttgttttttc tttttcgcta ttgtaaaatt 60  
ggagggggca aagttttcag ggtgttgtt agaatggaa gatgtccctt gtatcacat 120  
ggaccctcat gataatttg ttctttcac ttctactct gttgacaacc attgtctcct 180  
cttattttct ttccattttc tggtaacttt tcgtaaact tttagttgca 240  
attttaaat tcacttttgt ttatttgtca gattgttaagt acttctcta atcactttt 300  
tttcaaggca atcagggtat attatgtt attatgtt acttcaggac agtttttagag aacaatttgtt 360  
ataattaat gataaggtag aatattctg catabaaatt ctggctggcg tggaaatatt 420  
cttattggta gaaacaacta catcctggc atcatcctgc cttctctttt 39  
tgatatacac aaccatgttc tggatggat gaggataaaa tactctgagt cccctctgct 480  
aaccatgttc atgcctttt ctttttccctt ccaaacggg 540  
576

## FIG.2

Nucleotide Sequence of the  $\beta$ -globin intron II

gtgaggttgg ggacccttga ttgttcgtttc ttgttcgtttc ttttcgctta ttgtaaaatt 60  
ggagggggca aagtttcag ggtgttgtt agaatggaa gatgtccctt gtatcacat 120  
ggaccctcat gataattttg ttctttcac ttctactt ttgttccat 180  
cttattttct ttccattttc tgtaacttt tcgtaactt ttgttaacca 240  
atttttaat tcacttttg ttattttca gattgttaagt acttctcta atcactttt 300  
tttcaaggca atcagggtat attatattgt acttcaggcac agtttagag aacaattttt 360  
ataatttaat gataaggtag aatattctg catataatt ctggctggcg tggaaatatt 420  
cttatttggta gaaacaacta catcctggtc atcatctgc ctttctttt atggttacaa 480  
tgatatacac tggtttagat gaggataaaa tactctgagt ccaaaccggg cccctctgct 540  
aaccatgttc atgccttctt ctttttccata cag 573

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**FIG.3A**  
Eco RV-SpeI Fragment of Semliki Forest virus replicon

FIG.3B

tcccgaga ccgtgctcaa gaggccaaag ttggccccgg tgccaccctctt aggccaggcag 1560  
 gtgaaaataa taacacataa cggggggccc ggcggtttacc atatgacggc 1620  
 agggttcac taccatgtgg cggtccatt cgggtccctg agtttcaagg tttagggagg 1680  
 aggccacta tggtgtacaa cgaaggggag ttcgtcaaca gaaactata ccatattgcc 1740  
 gttcacggac actgacggcc gtcgctgaa caccgacgg gagaacctacg agaaaggta 1800  
 actgacggcg tcgggtttgg agtacgtgtt cgacgttagat aaaaatgtgtt cccatgaaatt 1860  
 gggctgaaga tcagggcgtc gcaaggctgtt gggccattat ggaccccggt aacccccacc 1920  
 cgggatcag tgggtttgg agagctaacc ggcaccatat tattattaag ggactacag aagactacag 1980  
 agccggaa gcaaggatcag tcaaggactt ccggaaaata gtaacgacg gttaaacacg 2040  
 atcctatacg ctggacgggg cttcgcttgc cattccggta cttcggttgc cttcggttgc 2100  
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 aatatgtcc agcttaaggt gaacttcaac gaaacttcaac cacaacatctt 2220  
 agtatacc gacgttgac accgttgac gcaaggatcgtt cggccatcc 2280  
 agtatacc gacggatggc accgttgac gcaaggatcgtt cttccggta 2340  
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 cagtggact accgttgac accgttgac gcaaggatcgtt cttccggta 2640  
 aaaggggat gggccatcc gcaaggatcgtt cttccggta 2700  
 accaaatgc cggccatcc gcaaggatcgtt cttccggta 2760  
 cggccatcc gcaaggatcgtt cttccggta 2820  
 gaaaggatggc gggccatcc gcaaggatcgtt cttccggta 2880  
 gtggacggcg tccaggaaacaa gaaaggatggc gcaaggatcgtt cttccggta 2940  
 gagggacggat gggccatcc gcaaggatcgtt cttccggta 3000  
 gttggacccgtt cttacttcc gaaatcgtt cttccggta 3060

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### FIG.3C

WO 99/25859

PCT/CA98/01065

cactgggata acagaccctgg tggaaaggatg tatggattca atgcccaac agctgccagg 3120  
ctggaaagcta gacatcacct cctgaagggg cagtggcata cggccaaggca ggcaggttatc 3180  
gcagaagaaa aaatccaaac ccctgtggc gcttctgt ctggacaatg taattccatat caaccggcagg 3240  
ctgcccggc acgttacaatg tgtagtacaatg ccacgttcctg acggttaaag gcagtagggg tgaggtaggtg 3300  
gtcaataaag taaggggta ccacgttcctg ctggtagtg agtacaacct ggctttgcct 3360  
cgacggagg tcacttggt gtcaccgtg aatgtcacatg gcccggatag gtgctacgac 3420  
ctaaggtag gactggggc tgacggggc aggttcgact tggtcttgtt gacatccac 3480  
acggaaattca gaatccacca ctaccaggc ctaccaggc actgctaaaa cccggggca tcttgatgag agcttacgga 3540  
cttggggag atgcgttacg aatcaggcga agccgttgt tcctccctaa gcagaaaagt tttctccaaac 3600  
tacgggata aatcaggcga agccgttgc tgtcaccagg ctctacgcta aatcagaag tgttcttgct 3660  
agagtgtgc gcccggatg gaaaggagacc caccaggatga caccaggat cttacaggat 3720  
tttgacaacg tatggggatg aagccatgca cgtgcacaga cttacggccgt 3780  
tatggatggc gaaaggagacc gaaaggagacc caccaggatga caccaggat 3840  
gacatggccca cgtgcacaga aagccatgca cttacggccgt 3900  
gacatggccca cgtgcacaga aatcggccgt ctaacggcag cggccttaa 3960  
ggggatggc aatcggccgt accccgtcat ccacgctgtt 4020  
acaccgtgg gacaaattaa tttcgccac tttcgccat gcgaaattggc cgctgtctac 4080  
ggggatggc acaccgtgg gcacaaattaa tttcgccac tttcgccat cccgctgtt 4140  
ggggatggc acaccgtgg gcacaaattaa tttcgccac tttcgccat ccatctattc 4200  
ggggatggc acaccgtgg gcacaaattaa tttcgccac tttcgccat cccgctgtt 4260  
acaccgtgg tttcgccac tttcgccat aatccctcaa gcgtagccat gcaatctcaa aatccctcaa 4320  
acaccgtgg tttcgccac tttcgccat cccgctgtt 4380  
acaccgtgg tttcgccac tttcgccat aatccctcaa gcgtagccat gcaatctcaa aatccctcaa 4440  
acaccgtgg tttcgccac tttcgccat cccgctgtt 4500  
acaccgtgg tttcgccac tttcgccat aatccctcaa gcgtagccat gcaatctcaa aatccctcaa 4560  
acaccgtgg tttcgccac tttcgccat cccgctgtt 4620  
acaccgtgg tttcgccac tttcgccat aatccctcaa gcgtagccat gcaatctcaa aatccctcaa 4680

## FIG.3D

gcagaacgga tcgcccggcct taggtcacac caagtaaaaa gcatgggttgt ttgctcatct 4680  
 ttccctcc cgaatacca ttttagatggg gtgcagaagg taaaatgcga gaagggttctc 4740  
 ctgttgacc cgacggtac ttcaagtggtt agtccgggaa gacttgact atctacgacg 4800  
 gaccactcag atcggtcggt acgggggtt gacttggact gggaccacca ctcgtcttcc 4860  
 actgcccagg ataccatgtc gctaccaggtt gtcaggatcgat gtgacatcga ctcgatctac 4920  
 gagccaatgg cttccatagt agtgacggct gacgtacacc gacgtacacc tgAACCTCGA 4980  
 gacctgggg cagatgtgca ccctgaaccc gcggccccc aactggccatg ggACCCGATT 5040  
 cttccaccgc gcccggac gaaaggccgac gcacgagggtc gatgggttgg cttggggat 5100  
 acgttccggc actttgacra gctgggttggc ggggggtcat atatttctc cttggacact 5160  
 gacttcgacg acgttcgtcg actaggccc ggggggtcat atatttctc cttggacact 5220  
 ggcaggccac attacaaca aaaatccgtt aggccacaca atctccaggat cggacacaactg 5280  
 gatgggtcc agggggggaa aatgtacccg ccaaaaattgg atactgaggag ggAGGAAGCTG 5340  
 aaatggcagat gaccccatcg gaggctaata agagtcgata ccagtctcgcc 5400  
 acatggggccgaaatggcgtt aatggggatggg atactggggatggg gggggggatgggg 5460  
 ttgctgtcgta aatgtggatgc gacgggttca catcgggggc cagattgtac 5520  
 aaggtaggaga aatgtggatgc gacccatcg gaggcttaata agagtcgata ccccggttac 5580  
 ttccctaccg acgttagggcg gacgggttca catcgccggc ggttaccccg 5640  
 taccttatcca gaaattaccg acgttagggcg gacgggttca ataccaaac 5700  
 tacttggaca tggttggacgg gtttggatagg aacagtggcg ttgttggaca 5760  
 aagttccggt gtttggacgg gtttggatagg gtcggatagg acatcatcg 5820  
 ccgttaccct ttcaaggacac actacaaac actacaaac 5880  
 aacgttaccc aatggggatggg actacccac actacaaac 5940  
 ttcaaggatcg atggactcg gggaggatat ttcaaggatcg actacccac 6000  
 ataaccactg ttgttccgg atggactcg accaaatttga aaggcccgaa 6060  
 gtcgacatga ttgttccgg atggactcg accaaatttga aaggcccgaa 6120  
 aacacacaga ttcccattgg aacacacaga ccaggggacga 6180

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## FIG.3E

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aaagtccagg	taattcaagg	ttggcgaccg	cttacctgtg	cggcatccac	6240
agggaattag	taaggaggact	aatatgtgtg	acgtgcacac	attgttttgat	6300
atgtcggccg	aagacttttg	cggatctact	tccacccagg	agaccgggtt	6360
ctagagacgg	acattggcatc	attcgacaaa	agccaggacg	tcttacagg	6420
ttaatgtatcc	tcgaaggatct	agggtggat	cagtacctgc	tgagacttgt	6480
tttggggaaa	tatccaggctg	tcacctacca	actggcacgc	tttcaagtt	6540
atgaaatcgg	gcatgtttct	gactttgttt	attaacactg	tttgaacat	6600
aggagggtac	tggaggcagg	actcaactgac	tccgcctgtg	cggccttcat	6660
aacatcgttc	acggaggatcat	ctccgacaag	ctgatggcgg	gtcggtgggtc	6720
aacatgggg	tgaaggatcat	tgacgctgtc	atggggaaa	tttttgtggg	6780
ggattcatag	tttttgcaga	acgcgcgtcc	aacccccata	tttacttaag	6840
cgctgttca	agtgggttaa	cgtcacacag	gtgtttcaga	cccacttaag	6900
cgaggactga	gtgacggagg	ttccggacaa	gtgtttcaga	agacaggcga	6960
gtggcactaa	catctaggta	ttccggacaa	gtgtttcaga	agcaggacga	6960
accttggcga	gggacattaa	ttccgggtta	ttccggacaa	gtgtttcaga	7020
ggcggtccta	gattgggtcg	ttccgggtta	ttccggacaa	gtatccctat	7080
taggatccag	atccccggta	ttccgggtta	ttccggacaa	acacctctac	7140
ccgtgtccc	cgccgggtcc	ttccgggtta	ttccggacaa	cgccactatta	7200
cgtcggtccc	gacttccagg	ttccgggtta	ttccggacaa	tttacggccg	7260
gacaatgaga	cagaacggaa	ttccgggtcc	ttccgggtta	cggtggctcc	7320
aaccaaacca	aaggccggaa	ttccgggtcc	ttccgggtta	taaatgcgct	7380
gaagaaggat	aaaggccgg	ttccgggtcc	ttccgggtta	agaagaagac	7440
catgaaatgt	ttccgggtcc	ttccgggtcc	ttccgggtta	ggaaaaaacgc	7500
cagacatgtc	cgctatcgg	ttccgggtcc	ttccgggtta	ccacagtaac	7560
tcgcaatcgg	cgctatcgg	ttccgggtcc	ttccgggtta	gtatgtttc	7620
tttagggtag	caatggcatt	ttccgggtcc	ttccgggtta	ctcggggtgg	7680

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FIG.3F

gcaatggcat	ataaccataa	ctgttataact	tgttaacaagg	cgcggccaaat	7800
tggcccggtg	gtccgcctca	cggaaactcg	tttaattggca	tattgacaca	7860
ataattggaa	gccttacataa	gcttaatttcg	ttttttttgc	ggatttttat	7920
aattggttt	taatatttcc	aaaaaaa	aaaaaaa	aaaaaaa	7980
aaaaaaaaa	aaaaaaaaa	aaaaaaaaa	aaaaaaaaa	aaaaaaaaa	8010

**FIG. 4A**

## Nucleotide sequence of pSFVlink

ttccaggcttc ttccaggcttg aaagatttgc cacgacgcca cccacgatgg  
ggatttacca cccacgatgg ccgccaagg catttccgtc ttgcagaagg  
catcaaggcttca aatgtaccat gcaaattgcca gggcatttc  
tcgaggcaggaa gacttgatgtc tagctacgca aaaaatttacc  
aaaggctcgaa agatcgagg ctaccttttgg  
aggacgtgtatggacggctga ttggattttgg  
aactggccaca ctacggccaca acatccaaac  
gacttgtgtc accatccgttgc  
agcaatttggaa ccttttaccc  
ggagaaggcttca ttagtccgtat  
ggccctgttac  
gttcccttagt  
ggccatcttaccc  
gatcccttagt  
ggccatcttaccc  
aaacacttaac  
tttagcaaggat  
gagagagggtt  
tgttacaaaggaa  
ttccaggatttt  
aactcgatcc  
accggacacc  
cggacaaatgg  
ttccaggatgg  
ttccaggctcc  
tgcccaccc  
60  
120  
180  
240  
300  
360  
420  
480  
540  
600  
660  
720  
780  
840  
900  
960  
1020  
1080  
1140  
1200  
1260  
1320  
1380  
1440

FIG. 4B

## FIG.4C

tatcaaacat tccacagggt aactttacgg ccacatggta agaaatggca gaagaacacg 3060  
 aaaaaataat gaagggtgatt gaaaggaccgt ctggcgctgt ggaacgcgttc cagaacaagg 3120  
 cgaacgttg tggggcgaaa agcctgggtgc ctgtccttggta cactgcccga atcagatgttga 3180  
 caggaggaa gtggaggcac ataaattacag catttaaggta ggacagaggct tactctccag 3240  
 tggtggcctt gaatgaaait tgccaccagg actatggagt tgacctggac agtggccctgt 3300  
 ttctggccctt gaagggtgtcc ctgttattacg agaacaacca ctgggataaac agacccgtgtg 3360  
 gaaggatgtt aaggatcaat gccgcaaacag ctggcaggct ggaaggctaga catacccatcc 3420  
 tggaggatgtt gttggcatacg gccaaggcagg accggaggct gcccacccgc 3480  
 ttctgtgtctt ggacaatgtt attcctatca acggcaggct agtggctggt ctggccgtgtg 3540  
 agtacaaggac ggttaaaggc agtagggttg ctttgcctcg acggcagggtc acttggttgt 3600  
 acgtccctgtt acgtggatgtt gttcaacccgt gctacgacact aagtttagga ctggccgtgtg 3660  
 caccggctgaa tggatgtt gttcgacttg gtcttgttga acattcacac ggaattcaga atccaccact 3720  
 acggccggcag gttcgacttg ttgtggatgtt gccatggaaagg tggaggatgtt ggcgtacgac 3780  
 accaggcgttcc cggggggcatc ttgtatggagg cttacggata cgccgatataaa atcaggcgaag 3840  
 tgcttaaacc ctccttaaqg ttcttgcgtt cgtctggcaag agtgtttggc ccggatttgtg 3900  
 ccgttgtttc ttggatgttcc agaaaggttct tctccaaactt tgacaacggaa aaggagccct 3960  
 tcacccaggaa tacagaagggtg ttcttgcgtt gttgggggatgtt tgccgggaa gccatgcaca 4020  
 ctacggctaca ccagatgttga accaaggctgtt tacagaggatgtt ggtatggcgta tgccaggccg 4080  
 cggccgggtt ctccttaaqg ttgtatggagg ttgtggatgtt ggtatggcgta accagggggc 4140  
 tggcgaaggaa atggccatcc taacggcgtt aacggccgtt ggtatggcgta tgccaggccg 4200  
 cgtcatgtt cggctgtgtt ttggatgttcc cccgtcatcc cccgtcatcc accgtgttagc gctctaatttc 4260  
 tggcgaaggaa atggccatcc taacggcgtt aacggccgtt ggtatggcgta tgccaggccg 4320  
 cgtcatgtt cggctgtgtt ttggatgttcc cccgtcatcc cccgtcatcc accgtgttagc gctctaatttc 4380  
 ctgaaggcggaa aggggaccgc ggttggccggc gatattggccg ctgtctaccg ggcagtggcc 4440  
 acagactgtc actggaggcgc gtggccatcc cgtctgtgttcc cccgtcatcc accaggagggt 4500  
 gaaggatgtt gctgcaggaa atctattcac tccctcaacc agcaatggac gcaacggacg 4560  
 ctgacgtgac catctactgc agagacaaaa gttgggagaa gaaaatccag gaagccatttg 4560

## FIG.4D

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acataggac ggctgtggag ttgctcaatg atgacgttggaa gacttggttga 4620
gaggcaccc ggacaggcagc ctgttgtggtc gtaagggtcta cagtaccact 4680
tgtactcgta ctttgtgggt acgaaattca accaggctgc tattgtatgt gcaggagatac 4740
tgacgttgtg gcccagactg caaggaggcaa acaaactatgc atgcctatac gcgtctggcg 4800
aaacaatggaa caaacatcaga tccaaatgtc cggtaaacacgat ttcggattca tcacacactc 4860
ccaggacagt gcccgtctgg agtggatgtc cttggatgtca aaaaatggatc 4920
ggtcacacca agttaaaaggc atgggtggtt gctcatctt tcccttcgg 4980
tagatgggt gcagaaaggta aagtggcgaga tatgcccgtat ctacgacggta 5040
cagtgggttag tccggggaaag cttggactgg accaccgact cgtctccac tgccaggcgat 5100
gagggtttgtgat tccgggttttgcgtt gcatcgact gacatcgact cccatagtag 5160
tacccaggtt gcaatgggtt cgtacaccct gatccggcag cctggggca 5220
tgacggctga cgtacaccct gacccatgtg gacccgttcc tccaccggcgc 5280
ctgcaaccggc agaccatgtg gacccgttcc gacccgttcc 5340
ctgcataact tgcctccggc gacggggggc gacccgttgc 5400
ctgcccggaaact gactggcggtt aggaacaaggc tggcttttgc 5460
acggaggctcgat tgggtggatc tccggggatc ttgcggggac 5520
atgtccggccaaatgggttttttgcgtt cggacactgg cttcgacgac 5580
ttacaacaaa cacaactggg cacaactggg tgcggccat 5640
ttttctccct tccagggtgc agaaggctgtt ggtggggatc gggggggc 5700
ttccaggatcactt tccggggatc cacaactggg tgggttttttgcgtt 5760
ttccaggatcactt tccagggtgc agtgcgttccat gatggggatc gggggggc 5820
ttccaggatcactt tccagggtgc agtgcgttccat gatggggatc gggggggc 5880
ttccaggatcactt tccagggtgc agtgcgttccat gatggggatc gggggggc 5940
ttccaggatcactt tccagggtgc agtgcgttccat gatggggatc gggggggc 6000
ttccaggatcactt tccagggtgc agtgcgttccat gatggggatc gggggggc 6060
ttccaggatcactt tccagggtgc agtgcgttccat gatggggatc gggggggc 6120

```

**FIG. 4E**

FIG. 4F

FIG. 4G

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aagttttaaa	tcaattctaaa	gtatataatgaa	gtaaactatgg	tcttgacagtt	accaatggctt	9300
aatcagttag	gcacctatct	caggatct	cgtatcacggaa	tccatccatcg	ttatccatcg	9360
cccgtcgtg	tagataacta	gggcttacca	cggatccggaa	tcttgcccca	gtgttgcaat	9420
gataccggaa	gaccacgt	caccggctcc	gatattatca	gcaataaaacc	agccaggccgg	9480
aaggggccgg	cgcagaagg	gtcgcgttcc	tttatccggc	tccatccagg	ctattaatttg	9540
tgctacaggc	gttagtgtaa	gttagttcgcc	agttaatagt	ttgcgcacacg	ttgttgcctt	9600
ccaacgatca	aggggaggta	catgttgcgtc	gttttgtatg	ttttcattca	gttcggtttc	9660
cggtcctcg	atcggttgt	cacgctcggt	atcggttgt	aaaaaaagggg	ttagctccctt	9720
gtactcaacc	aatttcattt	catgtatccc	ttatcactca	ttatcactca	tggttatggc	9780
gtcaatacg	aagtccattt	ctgtcatgcc	gaagtaagg	tgctttctg	tgactgggtga	9840
acgtttctcg	atcgtttca	ctgttgcgtt	atccgttaaga	tgctttctg	cttgcccgcc	9900
acccactcg	gataataccg	cgccacatcg	atccgttgaa	ccgaggttgct	tcattggaaa	9960
gcaaaaaaca	ggggcgaaac	tctcaaggat	atccgttgaa	aaagtgtctca	gttcgatgtta	10020
aatactcata	ggggggccgg	tttacccatcg	tttacccatcg	tttctgggtg	tttctgggtg	10080
gagcggatac	atccggatcc	tttacccatcg	tttacccatcg	tttctgggtg	tttctgggtg	10140
tccccggaaaa	aaataggcggt	tttacccatcg	tttacccatcg	tttctgggtt	tttctgggtt	10200
ctgacacatcg	aaataggcggt	tttacccatcg	tttacccatcg	tttctgggtt	tttctgggtt	10260
acaaggccgt	tttacccatcg	tttacccatcg	tttacccatcg	tttctgggtt	tttctgggtt	10320
ggcatttagga	tttacccatcg	tttacccatcg	tttacccatcg	tttctgggtt	tttctgggtt	10380
tttacccatcg	tttacccatcg	tttacccatcg	tttacccatcg	tttctgggtt	tttctgggtt	10440
tttacccatcg	tttacccatcg	tttacccatcg	tttacccatcg	tttctgggtt	tttctgggtt	10500
tttacccatcg	tttacccatcg	tttacccatcg	tttacccatcg	tttctgggtt	tttctgggtt	10560
tttacccatcg	tttacccatcg	tttacccatcg	tttacccatcg	tttctgggtt	tttctgggtt	10620
tttacccatcg	tttacccatcg	tttacccatcg	tttacccatcg	tttctgggtt	tttctgggtt	10680
tttacccatcg	tttacccatcg	tttacccatcg	tttacccatcg	tttctgggtt	tttctgggtt	10740
tttacccatcg	tttacccatcg	tttacccatcg	tttacccatcg	tttctgggtt	tttctgggtt	10800

## FIG.4H

cgggcgtaga ggatctggct agcgatgacc ctgctgattg gttcgctgac cattccggg 10860  
tgtgcggaaacg gcgttaccag aaactcaga aaccaccga ggttcgtcca 10920  
agtttacgag agagatgata gggtctgctt cagtaaggcc aattaggtt 10980  
gtacatattg tcgttagaac gggctacaa ttaatacata accttatgta tcatacacat 11040  
acgatttagg tgacactata 11060

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## Construction of pSFVlink

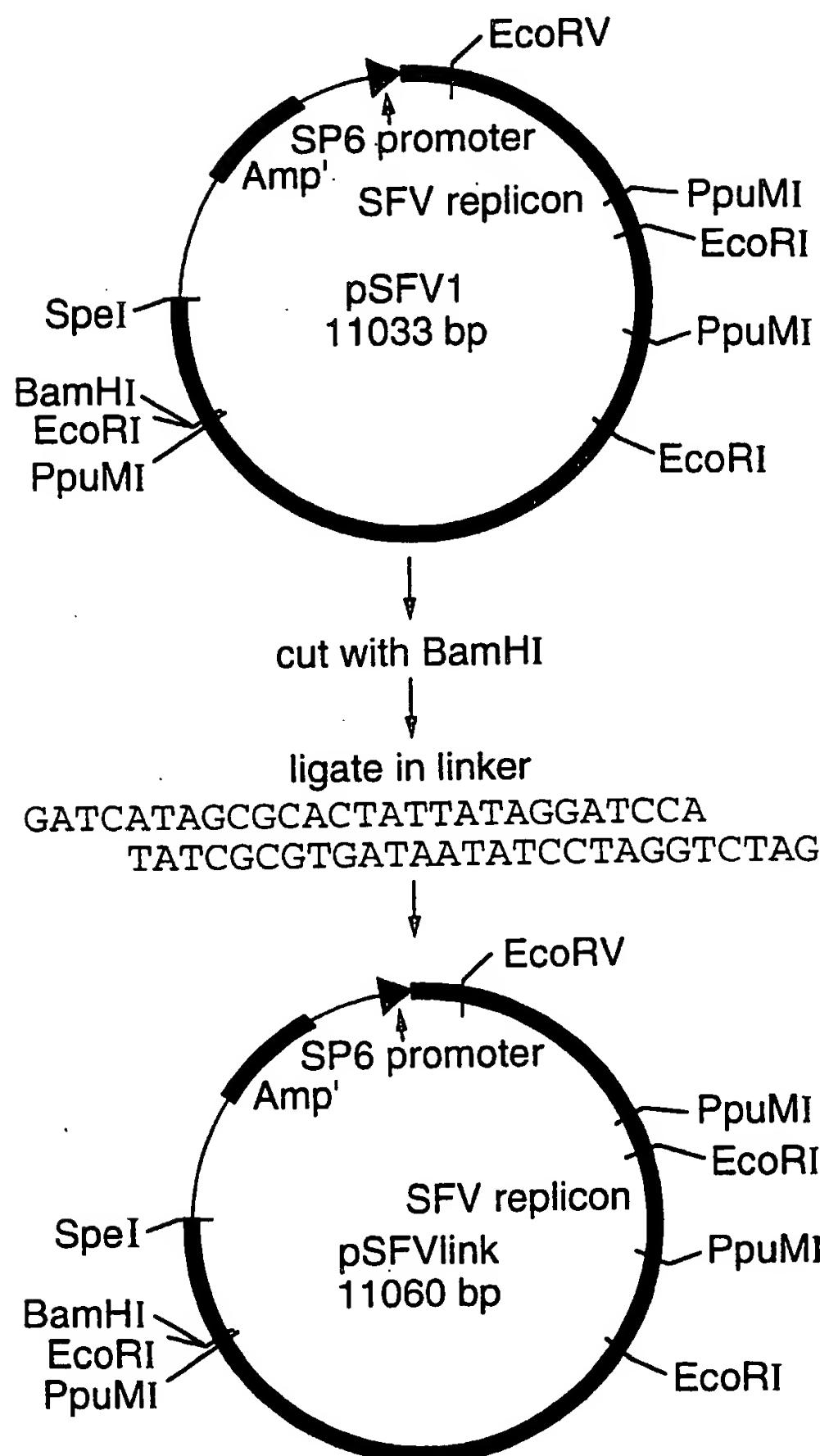


FIG.5

## FIG.6A

## Nucleotide Sequence of pMP76

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```

atggctatt ggcatttggca tacgttgtat ctagttacat ttatattggc 60
tcatgtccaa tatgaccggcc atgttgacat tggattttga ctatgtatca 120
attacggggt cattagttca tagccccat atggaggttcc gcgttacata 180
aatggccggc tcgtgacgg ccaacgacc cccgcccatt gacgtcaata 240
ttcccatagt aacgccaata gggacttcc attgacgtca atgggtggag 300
aaactggccca ttggcagta catcaagtgt atcatatgcc aagtccggcc 360
tcaatgtacgg taaatggccc gcctggcatt atgcccagta catgaccta 420
ctacttggca gtacatctac gtatttagtca tcgctattac catggtgatg 480
agtacaccaa tggcggtgg tagcgggttg actcacgggg attccaaatgt 540
ttgacgtcaa tggaggtttgg tttggcacc aaaatcaacg ggacttcca 600
ataacccggc cccgttgacg caaatggggc gttaggggtgt acgggtggag 660
gcagaggctcg tttagtgaaac cgtatggcg atgtgtgaca tacacgacgc 720
tgttccaggct cctgcccacct ccgctacgg agagattaac caccacgat 780
gtgcatgttg atattgggg tggaggtcatt ccaaatggacc ttcatcaagt 840
tcgttccagg ctaccaaatt gagactgaca ctttgagaaa 900
tcgcacctgg atcgccagg ctaccatggatc tctacggcaca 960
atcgccagg tggaggctg gagaatgtg tctacggcaca 1020
atcgccagg cagaaggaccc gaaaggctc gatagctacg 1080
tcgcacctgg cggctccagg gaaaggatcg gaaaaaatca 1140
atcgccagg cggccatggatc gggatcgca agagatcg 1200
tccggaaagg gctacggcc agcgttcaatc tcctaccc 1260
gctacggccagg ccggccgtata tggccgtata ccaggacgt 1320
catcaggcga tggaaagggtgt tattggattg tggatcgacac 1380
atgtttgacg cgctaggcagg cgggtatcca actacggca 1380

```

## FIG.6B

gtgttacagg ccaggaacat aggacttgtt gcaggcatcct tgactgaggg aagactcgcc 1440  
 aaactgtcca ttctcgca aacaccttttgcg acacagtcat gtttcggta 1500  
 ggatctacat tgtacactgaa gaggcagaagg ctactgagga atcccttacc accctccgtt 1560  
 ttccacactgaa aaggtaaacaa atccatgttgc tgtaggttgcg ataccatcg 1620  
 gggtagctggat ttaaggaaat cactatgtgc cccgggcctgt acggtaaaac ggttagggta 1680  
 gccgttgatgt atcaggcgaa gggatttccaa gttcaaaaggaa 1740  
 cttccgttgcg cttccgttgcg cttccgttgcg cttccgttgcg 1800  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 1860  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 1920  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 1980  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2040  
 19/39  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2100  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2160  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2220  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2280  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2340  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2400  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2460  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2520  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2580  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2640  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2700  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2760  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2820  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2880  
 gatccatgttgc gatccatgttgc gatccatgttgc gatccatgttgc 2940



FIG. 6

FIG. 6E

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atggacaaca	tca	atgtcccca	aacgattccg	attcatcaac	acctcccagg	6120
acagtgcct	gc	ctacgcaatg	acaggcagaaac	ggatcgcccc	cctttaggtca	6180
caccaagtta	aa	ggatgtgggt	tctttcccc	tcccgaaaata	ccatgttagat	6240
ggggtcaga	agg	cgagaagggtt	ctcctgttcg	accgcacggt	acattcagtg	6300
tttagtcgc	gga	cgcatctacg	acggaccact	cagatcggtc	gttagcaggg	6360
tttgacttgtt	act	cgactgtct	tccactgcca	gcgataccat	gtcgctaccc	6420
agtttgcact	cgt	gtgtgacat	cgactcgatc	tacgaggccaa	tggtctccat	6480
cccgcagacc	atgtggac	accctgaac	cggaggcatc	gcccgtgg	cggcagatgt	6540
ttaccttgcc	ccc	atgtggac	cggaaacccg	attcctccac	cgcgcgggaa	6600
ccaaggactg	gtc	gtcgatgcgt	ggaggcggacc	gtgcccggc	cgaggaaaggcc	6660
gtttaggcaggc	at	cgatctccgg	ggaggcggacc	ttgacgttcg	gcaacttgc	6720
atccatatttt	ca	caagctggcc	gattactttc	ggagactttc	cgacgttgc	6780
cgatattttt	tt	gattttaggaa	ctccctggac	actggcaggc	cgacttaggc	6840
gtttaggcaggc	tt	gtttaggcaggc	gtggccacaa	tccaggagga	gaaaatgtac	6900
atccatatttt	tt	gtttaggcaggc	gtggatggcg	ctggatggcg	gatggatgca	6960
cgatattttt	tt	gtttaggcaggc	ctgttgctgc	tgaaaatgca	aggcacccca	7020
ataccaggct	cg	ataccaggct	cgaaaagggtt	agaacatgaa	aggcacgggtg	7080
tcggaggcta	at	ataaggagtcg	ggccaggattg	ttcacggggag	cgagcgttgg	7140
gtttaggcaggc	at	tcacatcggt	tttccctta	ccgtgtatcg	ccaaacagtgt	7200
acatacggc	ac	acatacgg	tactccctta	ccagaaaatta	cgggtcggtat	7260
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gggttaccacc	tt	atgttttttt	ccatgttccat	ccatgttccat	ccaaacatcat	7500
aacgttgctag	tt	atgttttttt	ccatgttccat	ccatgttccat	ccaaacatcat	7560
accatggact	tt	atgttttttt	ccatgttccat	ccatgttccat	ccaaacatcat	7620
tattggaaag	tt	atgttttttt	ccatgttccat	ccatgttccat	ccaaacatcat	
gtgacccaa	tt	atgttttttt	ccatgttccat	ccatgttccat	ccaaacatcat	

三  
一  
六  
九

## FIG.6G

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```

ttgtggtcac ttgcatttggg ctccggcagat aagttagggt aggcaatggc attgataatg 9240
caaggaaatt gaaaacagaa aaaggtaggg taagcaatgg catataacca taactgtata 9300
acttgtaaca aagcgcaaca agacctgcgc acatttaattg gcaataattg gtggtccgcc 9360
tcggggcaac tcatttgac acatttaattg gcaataattg gaagcttaca taagcttaat 9420
tcgacgaata attggattt tattttatt tgcaattggg ttttaattt tccaaaaaaaaa 9480
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 9540
aaacgggtcg gcatggcatt cccacccctc tccacccatc cgccgggtccga cctgggcattc 9600
cgcacgtcca ctcggatggc taaggagat cctgaaactta acgctcgagt gcccaggccatc 9660
tgttgttgc ccctcccccg tgccctccct gaccctggaa ggtggccactc ccactgtccct 9720
ttcctaataa aatggggaaa ttgcattcgc ttgtctgagt aggtgtcatt ctattctggg 9780
gggtgggtcg gggcgggacca gcaaggggaa ggtttggggaa gacaataggca ggcatgtcg 9840
ggatgggtcg ggctcttagga tctcgaccat gcaagggttaag gatactgccc ggaacaaaac 9900
catgatcccg acggccatgcc agccctagtt tccagctttt gttcccttta 9960
catgatcccg atttcgagct taggtggagc tccagcttagt atggtcatacg ctgtttccctg 10020
tgtgggtta atttcgagct atttcgatcc tggcgtaatc acaacatacg aaaaaggaaaggc 10080
ttatccgctc acaaattccac tggcgtaatc tggcgtaatc aatccggccaa tcaactggccg 10140
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ttatccgctc acaatccac tggcgtaatc tggcgtaatc aatccggccaa tggcggttt 10620
ttatccgctc acaatccac tggcgtaatc tggcgtaatc aatccggccaa tggcggttt 10680
ttatccgctc acaatccac tggcgtaatc tggcgtaatc aatccggccaa tggcggttt 10740

```

FIG. 6.1

**FIG.6I**

**WO 99/25859**

**PCT/CA98/01065**

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tcggccctc gagcaagacg ttccccatg aatatggctc ataacacccc ttgttattact 12360  
gtttatgtaa gcagacagt ttattgttca tgatgatata ttttatctt gtgcaatgta 12420  
acatcaggaa ttttagaca caacgtggct ttccccccc ccccgagct tgat 12474

CMV promoter 1 - 682  
SFV replicon (before intron) 684 - 3678  
Rabbit (-globin intron II 3679 - 4251  
SFV replicon (after intron) 4252 - 9543  
Hepatitis Delta virus ribozyme (antigenomic) 9544 - 9628  
Kanamycin Gene 12342 - 11503  
BamHI site for insertion of heterologous inserts 8677

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Subcloning of the SFV replicon

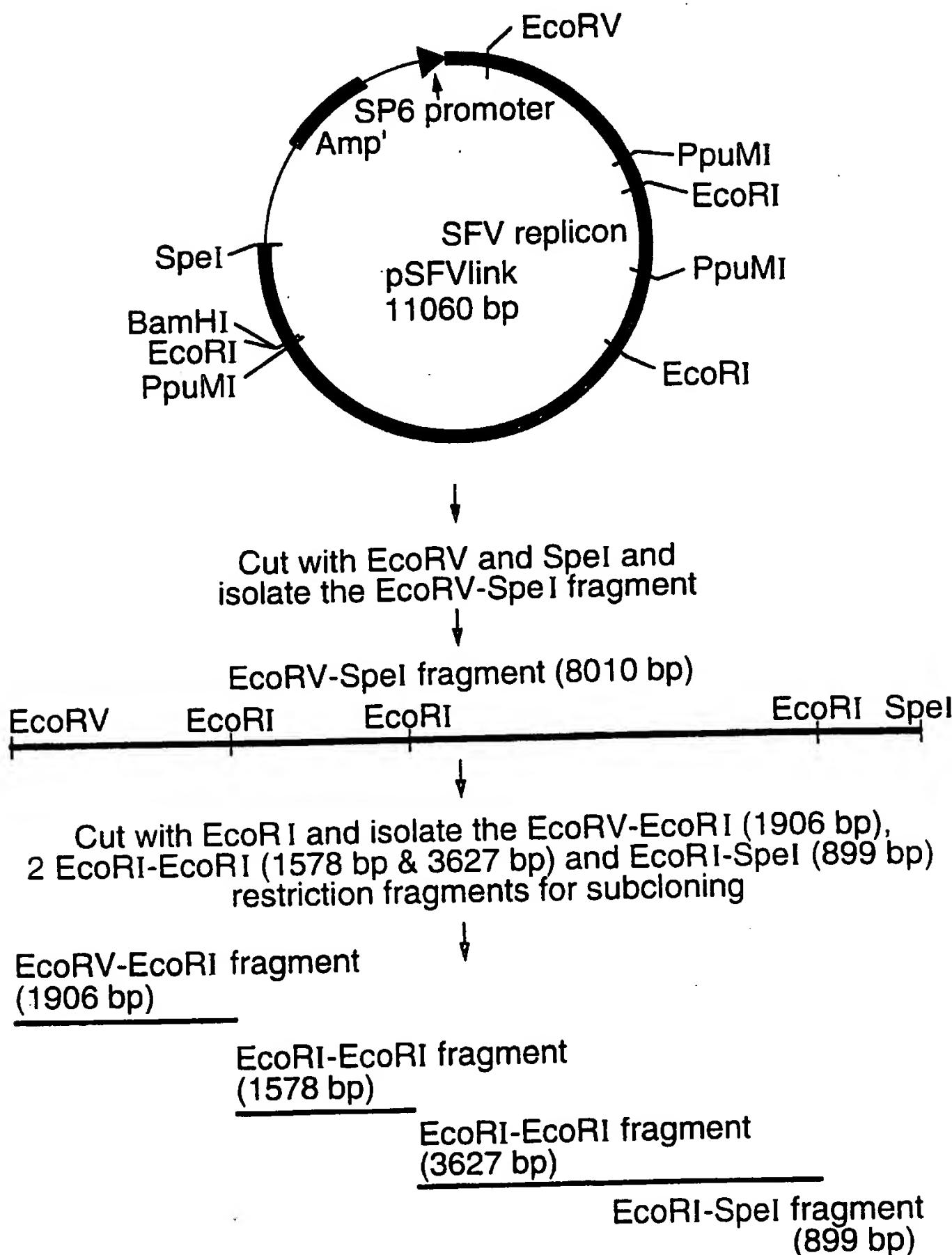


FIG.7

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## Construction of pMP76

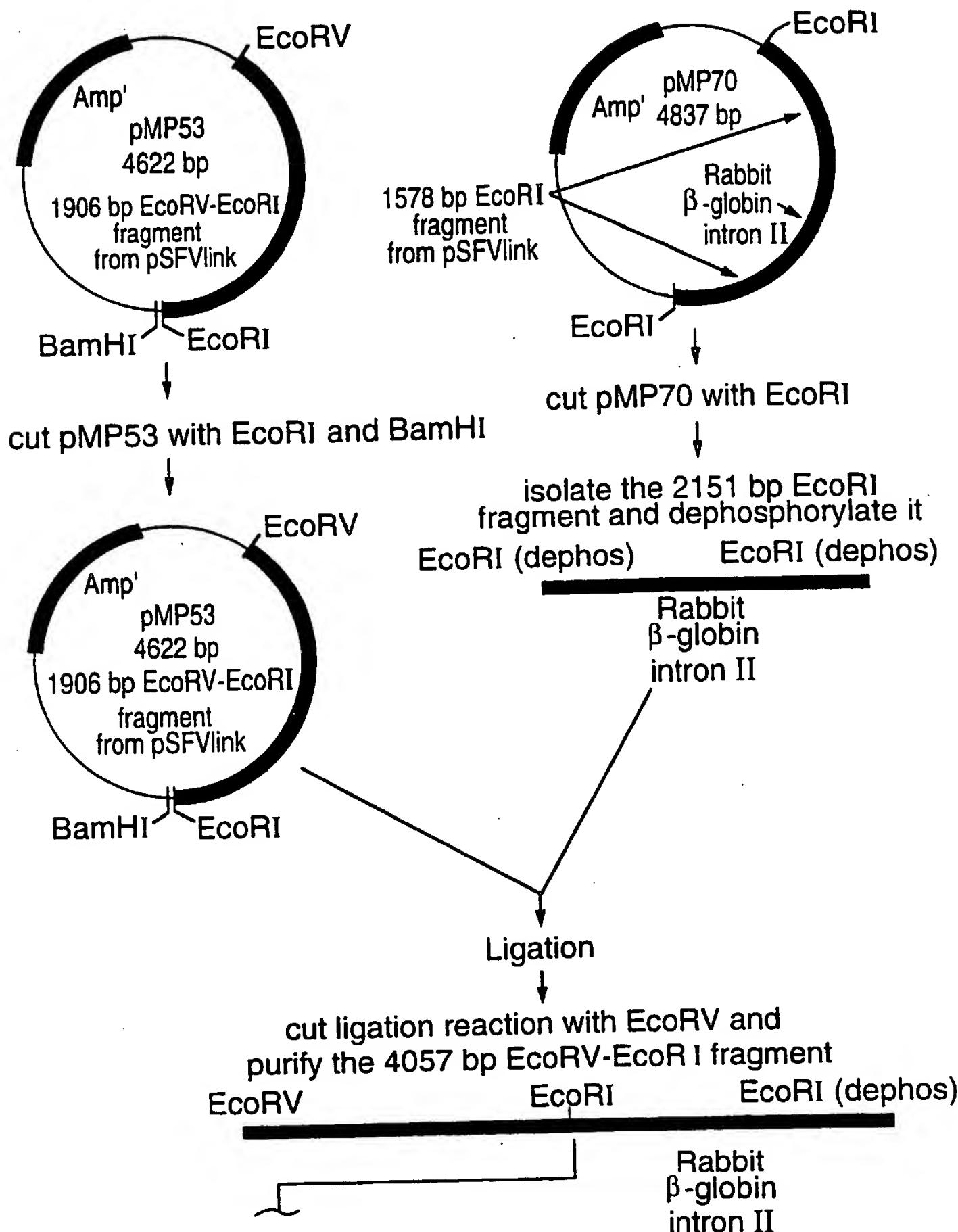


FIG.8A

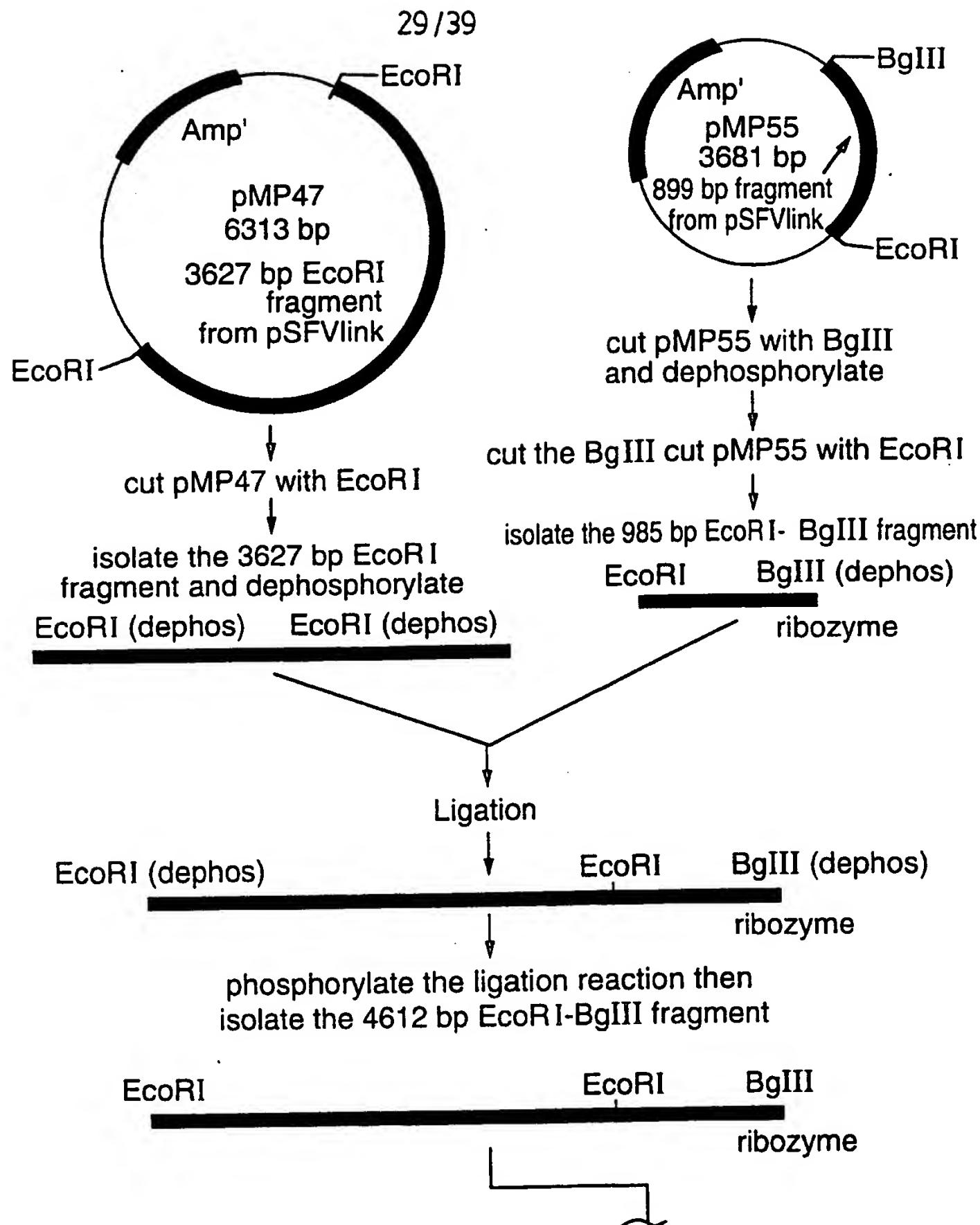


FIG.8B

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 Construction of pMP76

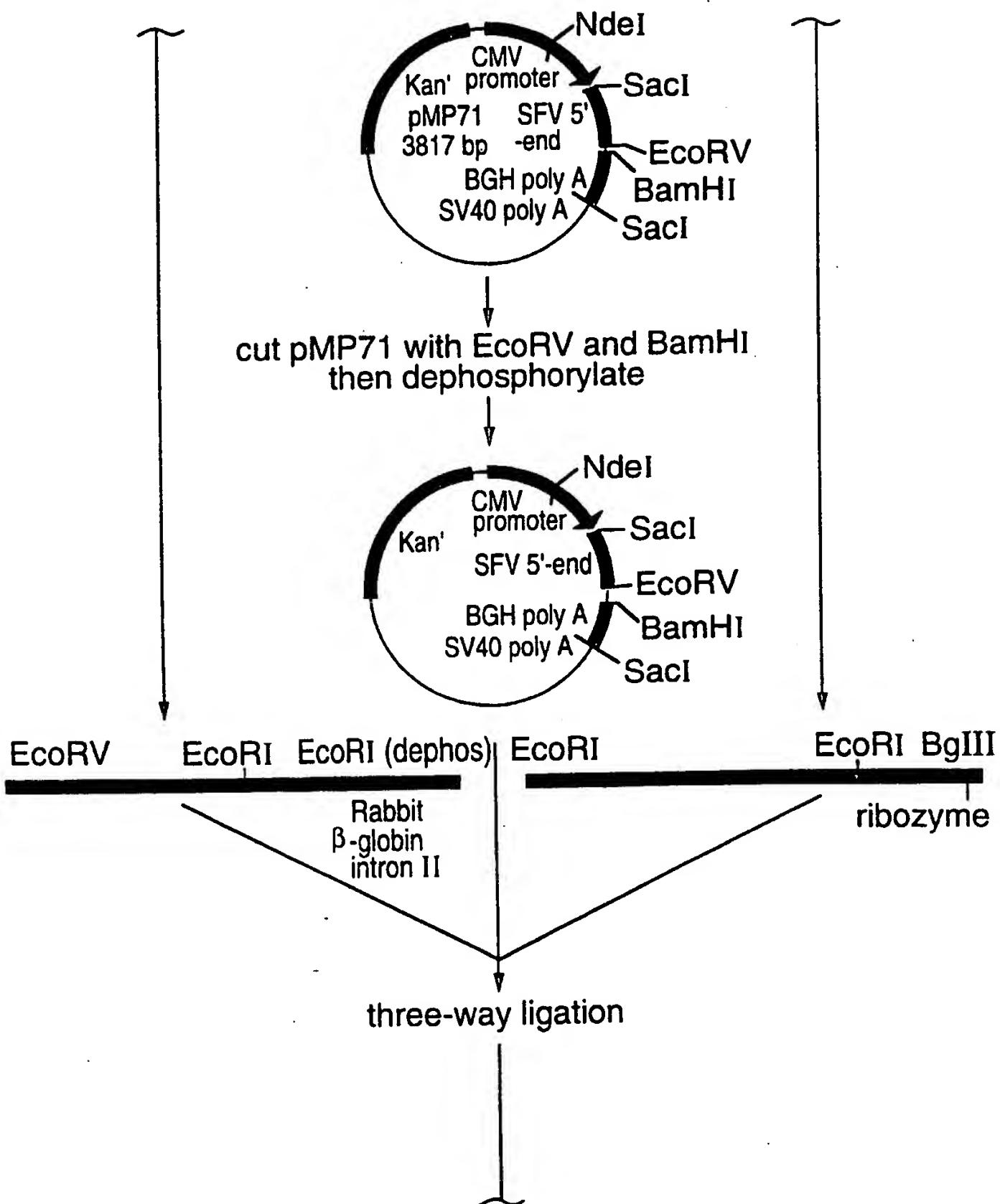


FIG.8C

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## Construction of pMP76 (cont'd)

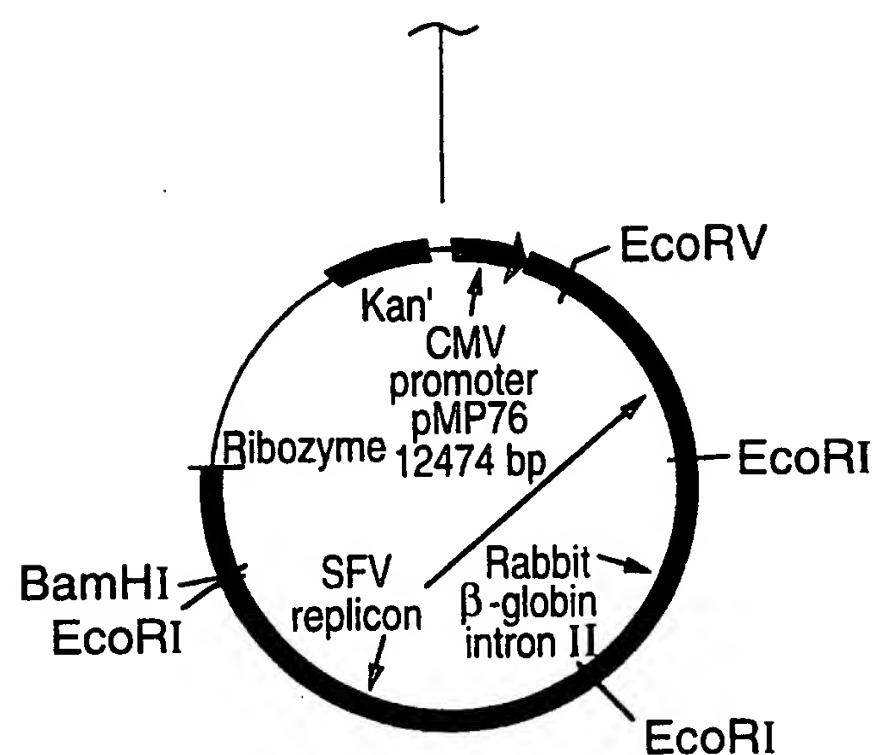


FIG.8D

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## Construction of pMP53 &amp; pMP54

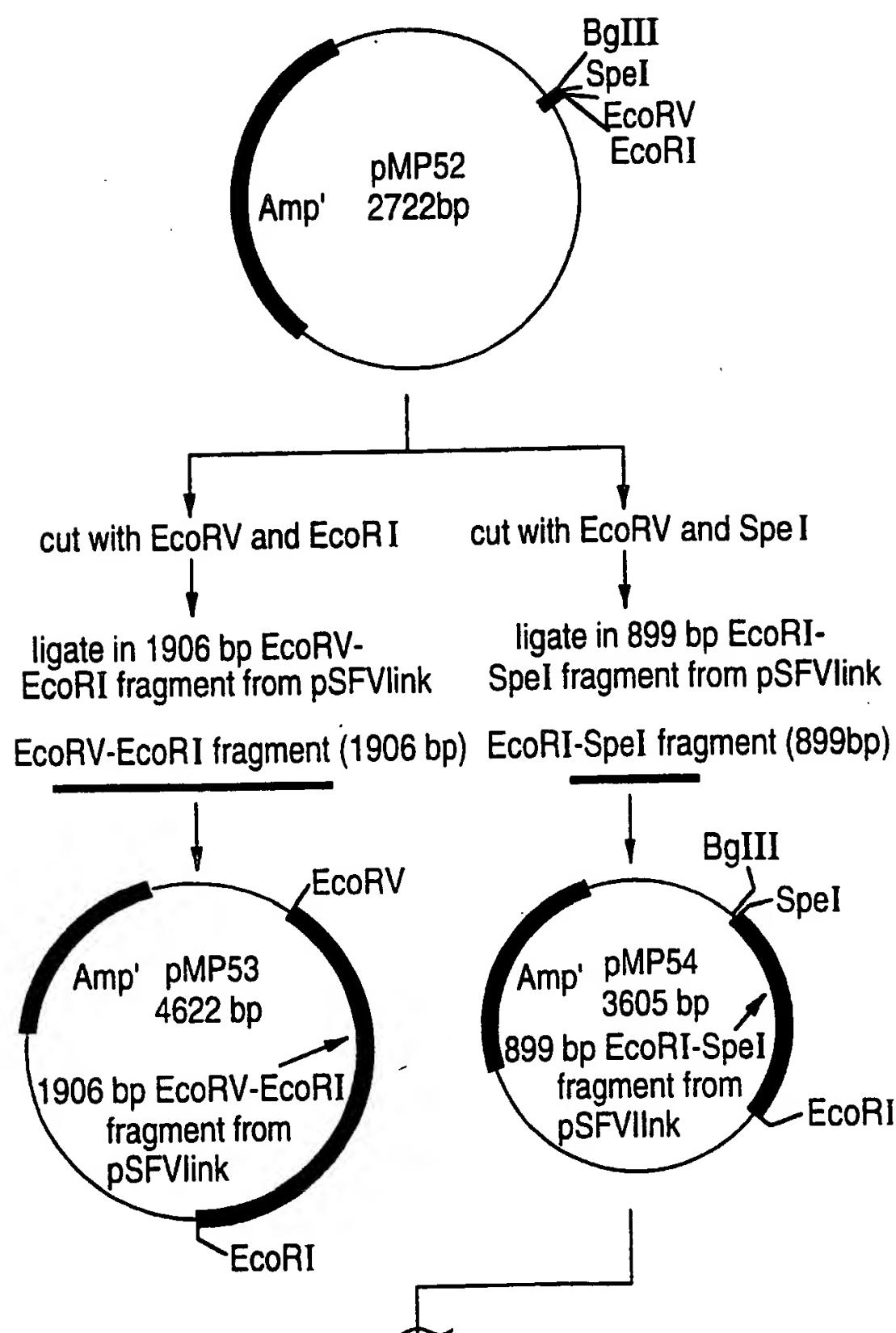


FIG.9A

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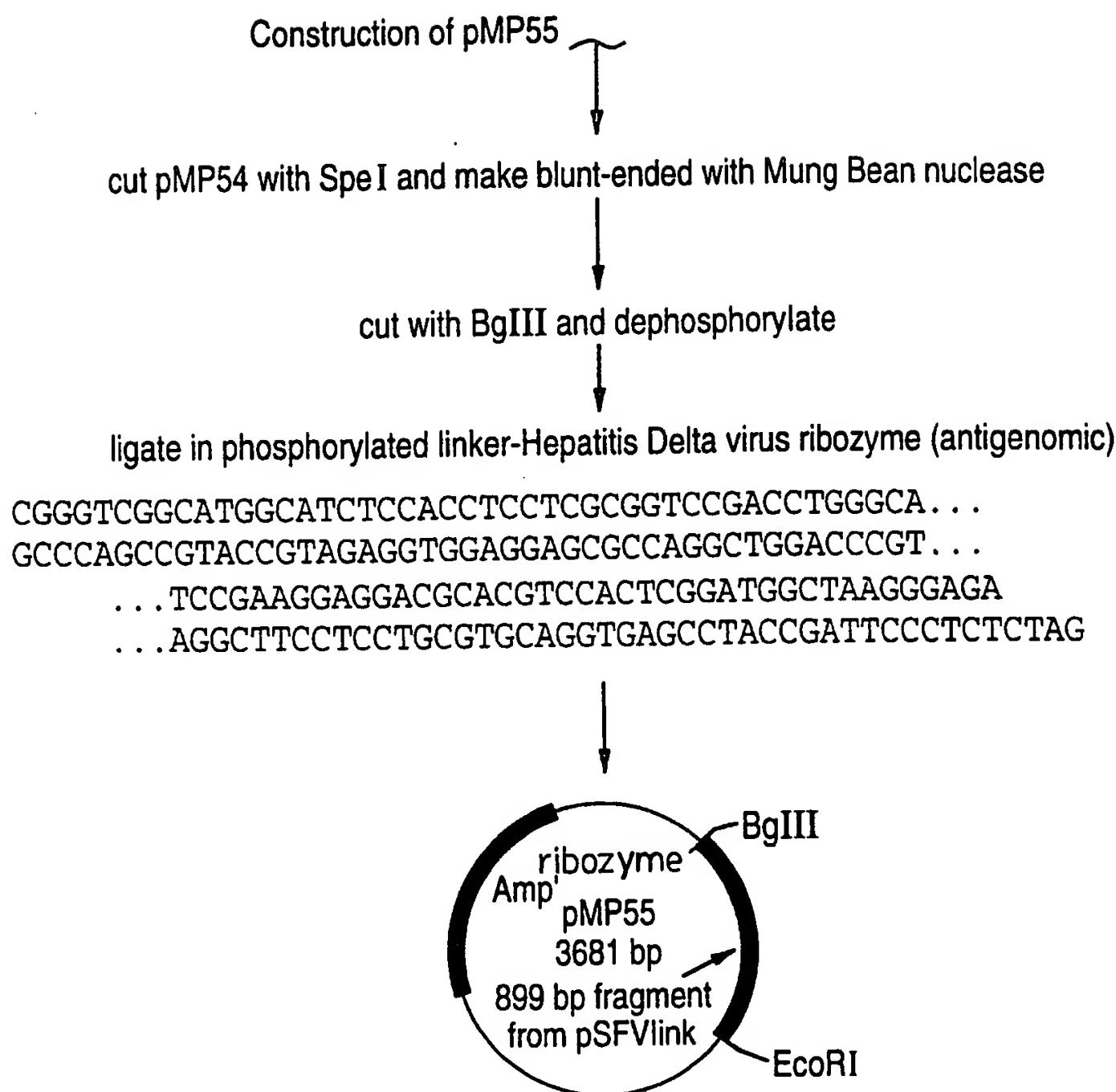


FIG.9B

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## Construction of pMP52

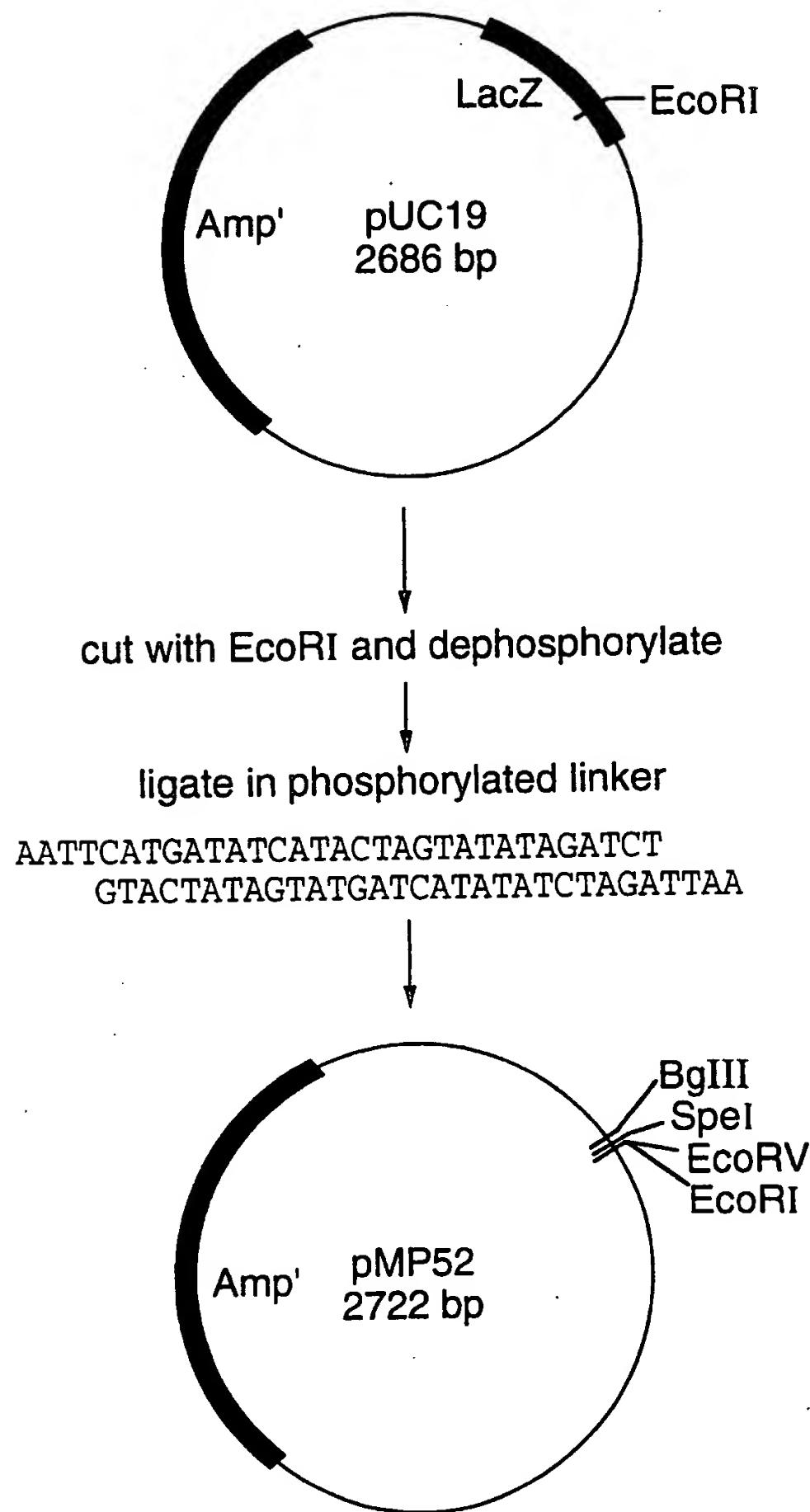


FIG. 10

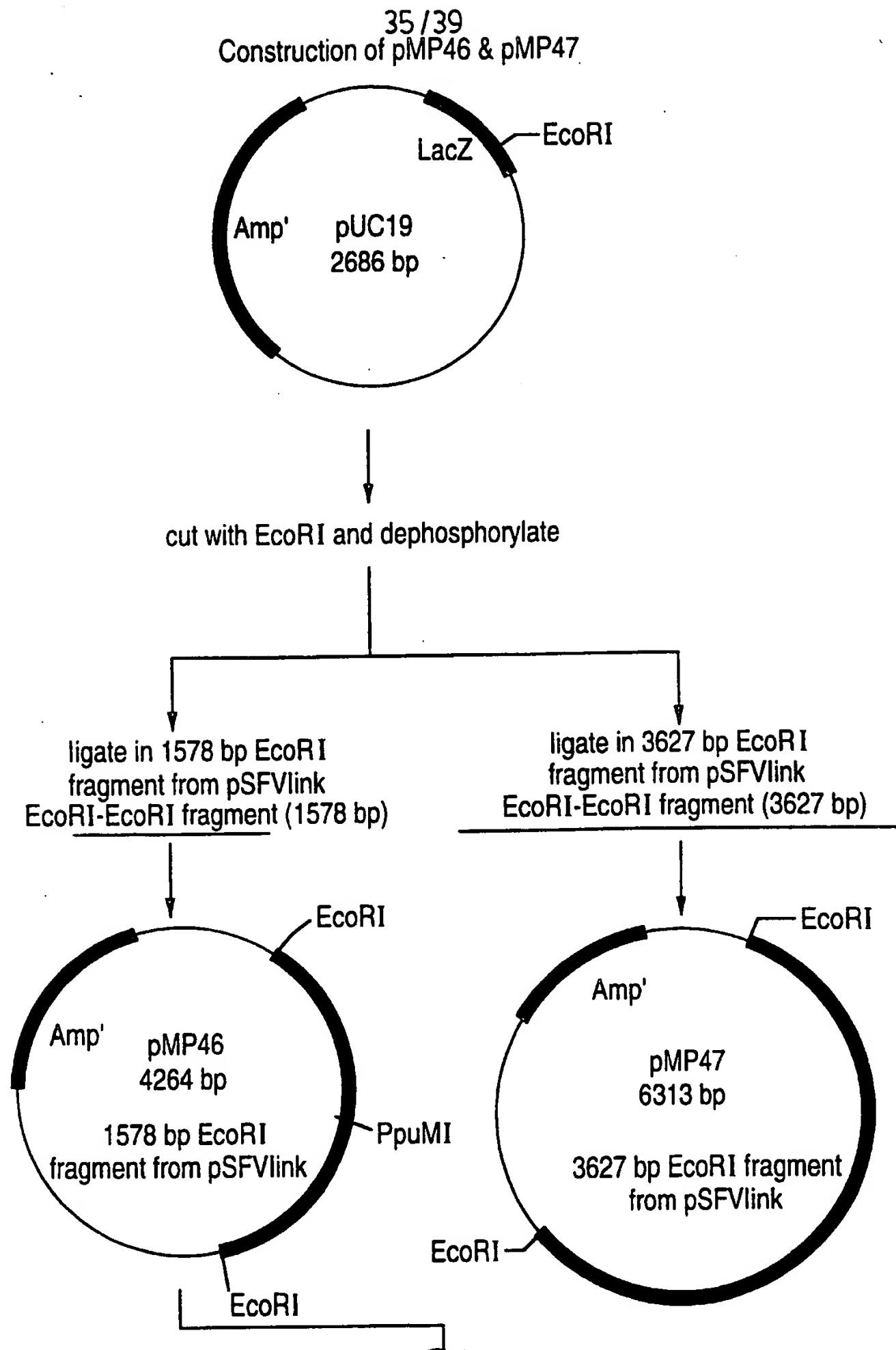


FIG.11A

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## Construction of pMP70

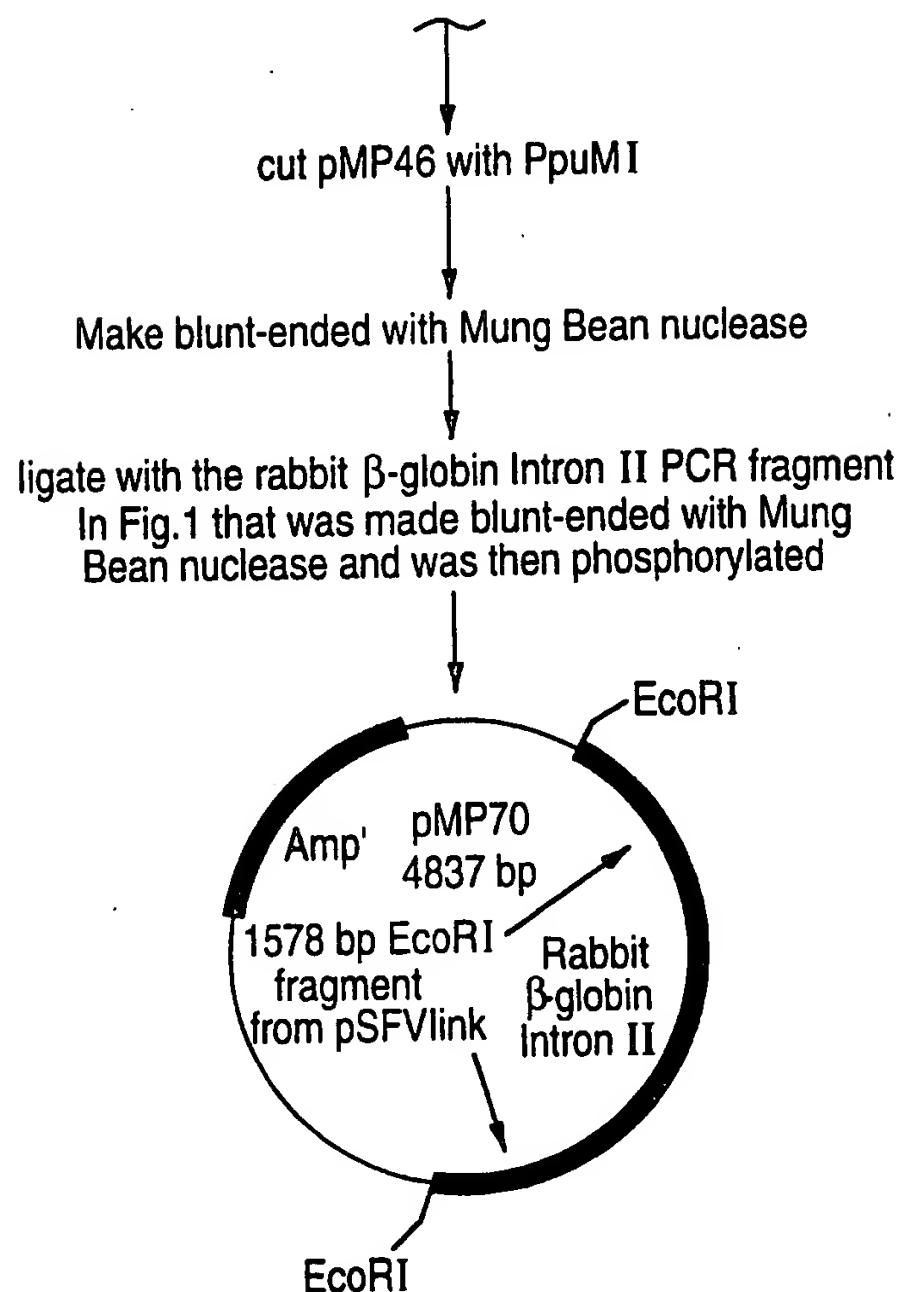


FIG. 11B

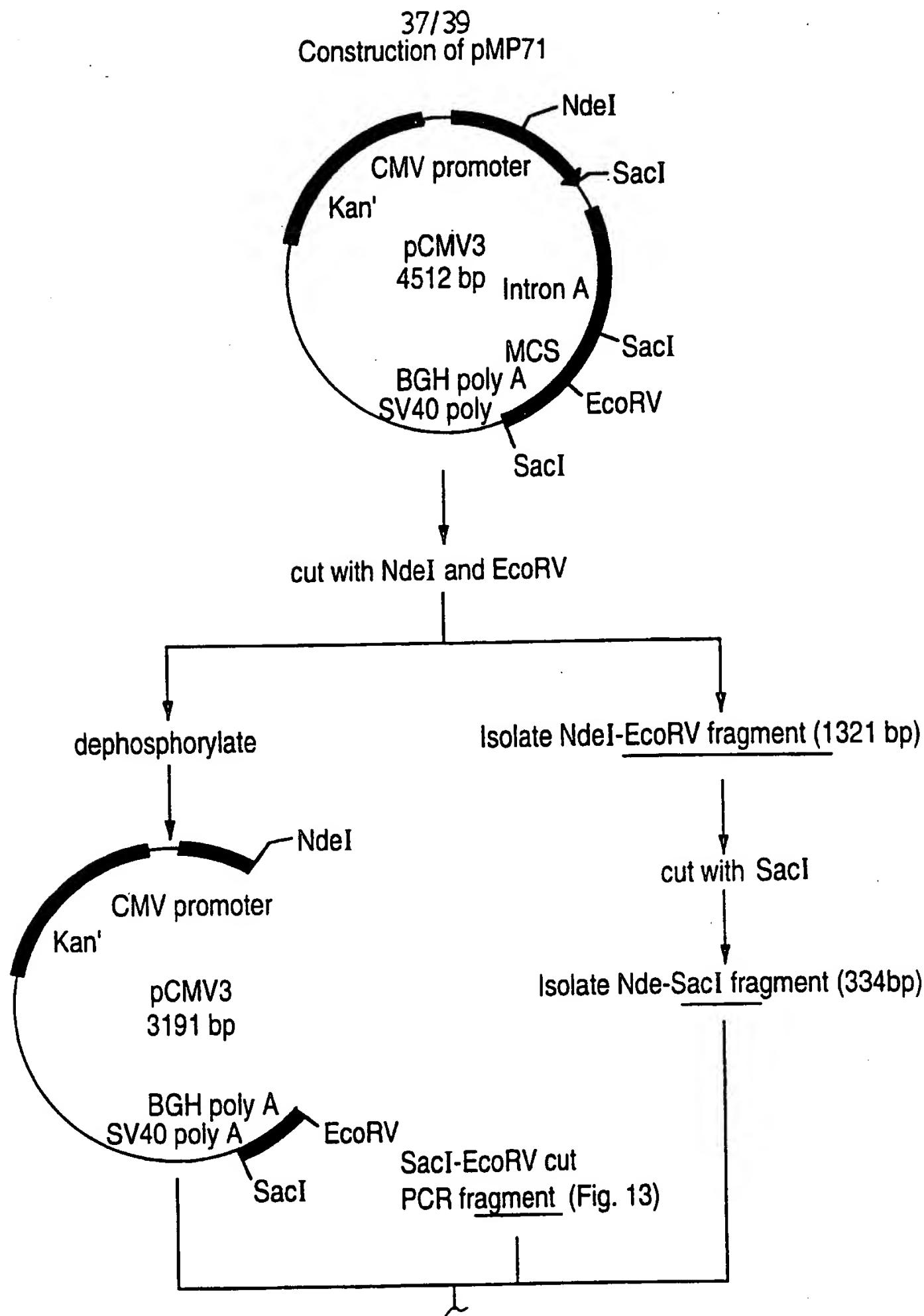


FIG.12A

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## Construction of pMP71 (cont'd)

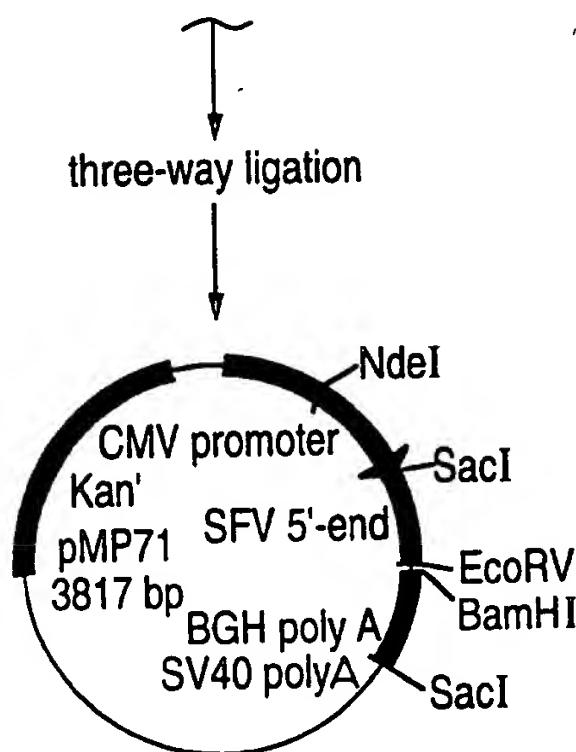


FIG.12B

## FIG. 13

1 CGTTTAGTGA ACCGTATGGC GGATGTTGTA CATAACGGAC GCCAAAGAT 50  
51 TTTGTTCCAG CTTCCTGCCAC CTCCGCTACG CGAGAGATT ACCACCCACG 100  
101 ATGGCCGCCA AAGTGCATGT TGATATTGAG GCTGACAGCC CATTCAAA 150  
151 GTCTTTGCAG AAGGCATTTC CGTCGTTCCA GGTGGAGTCA TTGCAGGTCA 200  
201 CACCAAATGA CCATGCAAAT GCCAGAGCAT TTTCGCACCT GGCTACCAA 250 39/39  
251 TTGATCGAGC AGGAGACTGA CAAAGACACA CTCATCTTGG AT 292

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/CA 98/01065

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 C12N15/86

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 27044 A (BIOPTION AB ;LILJESTROEM PETER (SE); GAROFF HENRIK (SE)) 12 October 1995 cited in the application see the whole document, especially page 8, lines 12-22 ---	1-14
Y	WO 96 40945 A (CONNAUGHT LAB ;LI XIAOMAO (CA); EWASYSHYN MARY E (CA); SAMBHARA SU) 19 December 1996 cited in the application see the whole document, especially page 6, lines 2-9; page 14, lines 15-21; and page 23, lines 18-23 ---	1-14
A	WO 96 17072 A (VIAGENE INC) 6 June 1996 see the whole document ---	1-14 -/-

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Patent family members are listed in annex.

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Date of the actual completion of the international search

23 April 1999

Date of mailing of the international search report

03/05/1999

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Mandl, B

**INTERNATIONAL SEARCH REPORT**International Application No  
PCT/CA 98/01065**C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZHOU X. ET AL.: "Self-replicating Semliki-Forest virus RNA as recombinant vaccine, VACCINE, vol. 12, no. 16, 1994, pages 1510-1514, XP002089524 cited in the application see the whole document -----	1-14
A	LILJESTROEM P. ET AL.: "A NEW GENERATION OF ANIMAL CELL EXPRESSION VECTORS BASED ON THE SEMLIKI FOREST VIRUS REPLICON" BIO/TECHNOLOGY, vol. 9, December 1991, pages 1356-1361, XP000616021 cited in the application see the whole document -----	1-14

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Appl. No
PCT/CA 98/01065

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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WO 9617072	A 06-06-1996	AU EP US US US	4594996 A 0797679 A 5814482 A 5843723 A 5789245 A	19-06-1996 01-10-1997 29-09-1998 01-12-1998 04-08-1998